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Energy Use During the Development of a Lecithotrophic and a Planktotrophic Echinoid

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Abstract. The energy required for development was measured in two closely related echinoids with differing modes of development. *Heliocidaris tuberculata* hatches from a 95-μm egg (~0.1 μg dry organic mass) and develops *via* a planktotrophic larva over 21–30 days into a juvenile (5.3–7.5 μg). *H. erythrogramma* hatches from a ~400 μm egg (11.6–19.0 μg) and develops over 3.5–4 days *via* a lecithotrophic larva into a juvenile with a mass not detectably different from that of the egg. Oxygen consumption increased exponentially in *H. tuberculata* and peaked at about 200–500 pmol indiv^{-1} h^{-1}, whereas the oxygen consumption of *H. erythrogramma* increased rapidly, reaching a plateau at about 800 pmol indiv^{-1} h^{-1} on the second day. Metabolic energy expenditure for development to metamorphosis was twofold higher for *H. tuberculata* (52–60 mJ indiv^{-1}) than for *H. erythrogramma* (26–35 mJ indiv^{-1}). The interspecific comparison suggests that about half the metabolic expenditure for planktotrophic development goes toward building and operating the larval feeding apparatus and that the return on this investment is 400%–600% over the larval period. When the energy equivalents of the organic masses of the juveniles are included, the energy for constructing a juvenile on a per mass basis is essentially the same for both species (*cf. H. tuberculata:* 37–42 mJ μg^{-1}; *H. erythrogramma:* 34–36 mJ μg^{-1}) and implies the absence of developmentally based energetic barriers or benefits to changes in modes of development. Substantial amounts of metabolically inactive material may be present in embryos with nonfeeding development and should be considered in physiological measurements and comparisons.

Introduction

Most marine invertebrates have life cycles that include a pelagic larva (Thorson, 1950) which may feed (planktotrophic) or not feed (lecithotrophic) on food particles. Nonfeeding larval development has evolved repeatedly in echinoderms and many other marine invertebrate phyla (*e.g.*, Strathmann, 1978a, 1993; Emlet, 1990, 1994; Wray, 1995) and is associated with increased size of eggs. With sufficient materials and energy in the larger eggs, lecithotrophic development is relatively rapid, and morphogenesis can be quite modified from that of related taxa with feeding larval development (*e.g.*, asteroids: Byrne, 1991, 1995; McEdward, 1992; Janies and McEdward, 1993; echinoids: Raff, 1987; Wray and Raff, 1989, 1990; nemerteans: Martindale and Henry, 1995). Among echinoids, studies on species with feeding and nonfeeding larval development have demonstrated remarkable changes in early embryology and larval form. Patterns of cell cleavage (Raff, 1987), cell lineages (Wray and Raff, 1989, 1990), and mechanisms of blastulation and gastrulation (Henry *et al.*, 1991; Wray and Raff, 1991; Schatt and Feral, 1996) have apparently changed. The timing of expression of larval and juvenile traits has been rearranged (*e.g.*, Raff, 1987; Wray and Bely, 1994; Emlet, 1995); larval shape and degree of retention of the ancestral larval structures varies (*e.g.*, Amemiya and Emlet, 1992; Olson *et al.*, 1993; Emlet, 1995; Morris, 1995). The length of the larval period and the size at metamorphosis can change (Lawrence *et al.*, 1984; McClintock and Pearse, 1986; Emlet *et al.*, 1987; Emlet and Hoegh-Guldberg, 1997).

Our understanding of how development evolves is particularly enhanced by comparisons between closely related taxa with differing modes of development. One area in which we lack such comparative data is the energy required for development. Energy required for development includes (a) the maternal energy contributed in the egg, (b) the total metabolic expenditure during development, and (c) the energy in the mass change between fertilization and metamorphosis. Larger eggs do

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contain more energy, but few studies have been able to provide precise estimates of the energy required for different developmental modes (Jaeckle, 1995). We know little about whether metabolism changes when development changes. Because lecithotrophic species have their own energy supply and usually develop relatively rapidly, how does their metabolic activity change after fertilization? If rates are adjusted for mass-specific metabolism, how do planktotrophs and lecithotrophs compare? The large yolky eggs of many lecithotrophs may contain metabolically inert materials; how might this confound interpretations of mass-specific metabolism? Do lecithotrophic larvae, which have a finite maternal energy supply, require more or less energy to produce a juvenile than is required by planktotrophic larvae that must gather materials and energy through feeding? Although the evidence suggests that nonfeeding development has evolved repeatedly and a considerable effort has been made to understand this in terms of life-history evolution (*e.g.*, Vance, 1973; Strathmann, 1985; Roughgarden, 1989; Havenhand, 1993, 1995), we know of no studies (or theories) that have searched for developmentally based energetic differences associated with particular modes of development.

The present study compares the energy use by two closely related species with differing modes of development. By providing several genera that include both planktotrophic and lecithotrophic species, the echinoderm fauna of southeast Australia presents an unusual opportunity to minimize evolutionary distance and environmental differences. The echinoid genus *Heliocidaris* includes two species that co-exist in the Sydney region. The adult distributions partially overlap in shallow, subtidal, rocky habitats, and in general both species experience similar thermal and other environmental regimens during their life cycles. Laegdsgaard *et al.* (1991) found different seasons of reproduction for the two species, but viable gametes of both species were readily obtained for this study during Austral summers. *H. tuberculata* (Lamarck) hatches from a small egg (diameter ~95 μm) and completes development *via* a feeding larva over 21–30 days at 22°C (this study). *H. erythrogramma* (Valenciennes) hatches from a relatively large egg (~400 μm) and completes development in 3.5 to 4 days at 22°C (Williams and Anderson, 1975; Raff, 1987; Emlet, 1995). The divergence time for the two species has been estimated as 5–8 million years, on the basis of differences in mitochondrial DNA (McMillan *et al.*, 1992), and as 10–13 million years, on the basis of DNA hybridization methods (Smith *et al.*, 1990). The small genetic distance (*ca.* 10 million years *vs.* 10's to 100's of millions of years), and the similarity of adult habitats and physical regimens of the life cycles between sibling *Heliocidaris* species minimizes the influence of factors unrelated to the comparison of developmental mode.

This study utilizes the close relationship yet contrasting developmental modes of sibling species of *Heliocidaris* to compare biomass changes and metabolism from fertilization through metamorphosis. With this information, differences in the energy budgets of a lecithotroph and a planktotroph are analyzed. We also take advantage of the fact that the blastocoelic lipid reserves of *H. erythrogramma* can be largely removed (Emlet and Hoegh-Guldberg, 1997) and use this manipulation to provide a perspective on the partitioning of energy use in the two types of development. Comparison between the two species of the total energy required for development yields a quantitative measure of the maternal contribution of energy required to produce a juvenile. This interspecific comparison also permits us to estimate of the cost of building and maintaining a larval feeding apparatus and its efficiency for accumulating the energy required to construct a juvenile echinoid.

Materials and Methods

Collection, maintenance, and culture of embryos and larvae

The experiments described here were conducted over three reproductive seasons (primarily December 1992–January 1993 and January–February 1994, but also January–February 1996). *Heliocidaris erythrogramma* and *H. tuberculata* were collected, under permit, from Shelly Beach, Fairy Bower, and Clovelly near Sydney, Australia. Adults were maintained at the University of Sydney in recirculating aquaria for up to a week. Animals were spawned by coelomic injection with 0.5 *M* KCl. Eggs were washed with several changes of 1-μm filtered seawater and fertilized with dilute concentrations of sperm. Fertilized eggs were placed in dishes without stirring for the first day, and gentle stirring of cultures began on day 2.

Embryos and larvae were cultured at 22°C and culture water was changed every 2–3 days. Cultures of *H. erythrogramma* were checked for juveniles beginning on the third day. Cultures of *H. tuberculata* were fed algal cells (*Chaetoceros gracilis* or *Rhodomonas lens*, CSIRO, Hobart) at final concentrations of 20–50 × 10^3 cells ml^{-1}, and the first individuals completed development in 21–30 days. Larvae of both species either metamorphosed spontaneously or were induced to metamorphose with scrapings from algal-encrusted rocks.

Removal of blastocoelic lipid from embryos of Heliocidaris erythrogramma

Lipid-rich vesicles are extruded into the blastocoel of *H. erythrogramma* during the first 8–12 h of development and prior to hatching (Henry *et al.*, 1991). These blastocoelic vesicles were removed from newly hatched

blastulae (12–14 h post-fertilization) by centrifuging blastulae for two 15-s intervals in a microcentrifuge (Beckman Microfuge E). Blastocoelic lipid extrudes through tiny rips in the blastoderm of the blastulae during centrifugation. Embryos at this stage do not have mesenchyme cells and hence the loss of cells from the blastula is minimal. This manipulation removes almost all the blastocoelic lipid, which occupies about 40% of the volume of the blastula (Emlet and Hoegh-Guldberg, 1997), and embryos treated in this manner develop normally to the juvenile stage in the same time as untreated larvae (Emlet and Hoegh-Guldberg, 1997). We refer to this as the "reduced-lipid" treatment.

Dry organic mass

Dry organic mass was measured for eggs, embryos, and larvae of the two *Heliocidaris* species at various developmental stages. These measurements were used to follow changes in biomass during development and to make calculations on energy use. Five or six replicate determinations of biomass based on 15–200 (*H. erythrogramma*) or 300–2800 (*H. tuberculata*) individuals per determination were made for eggs, embryos, and larvae. Individuals for organic-mass determination were concentrated by removing seawater around the embryos or larvae. Initial samples were frozen in small amounts of seawater, but later (and most) samples were rinsed for a few seconds in distilled water (McEdward, 1984) prior to being frozen (−20°C). Frozen larvae were added to small aluminum dishes (pre-ashed at 458°C for 6 h) and were dried at 80°C to constant mass (which occurred between 5 and 10 d). Samples were then weighed (total dry mass) to the nearest microgram on a mass-calibrated electrobalance (Cahn 25, Cahn/Ventron), and subsequently ashed at 458°C for 6 h. Ashed samples were weighed (ash mass) on the same balance, and dry organic mass was calculated as the difference between the total dry mass and the ash mass, and expressed as dry organic mass per larva.

Polarographic respirometry of growing and differentiating larvae

Polarographic respirometry was used to measure the metabolic rate of embryos and larvae at various stages during development and for embryos in which lipid reserves had been removed. Microrespirometry chambers (100-μl and 500-μl) and polarographic oxygen electrodes (Strathkelvin Instruments, Glasgow, U.K., OM780 and SI1302) connected to a data acquisition and analysis system (DATACAN IV, Sable Systems, Los Angeles, CA) were used to measure the metabolic rate of the embryos and larvae. Chamber temperature was 22°C and was controlled to within ±0.1 C° by a temperature-controlled water bath (HAAKE 6) connected to the water jacket of each microrespirometer cell. Respirometry chambers contained known numbers of individuals (range 5–175, usually 10–30 for *H. erythrogramma*, and range 20–1179, usually 50–150 for *H. tuberculata*) for each measure of oxygen consumption. The highest numbers were for eggs and cleavage stages, which had low rates of oxygen consumption, and the lowest numbers were for larval stages. Metabolic rate was calculated as oxygen consumption in pmol O_2 individual^{-1} h^{-1}. Specific metabolic rates (SMR = metabolic rates standardized to mass) were calculated by dividing respiratory rates by the mean dry organic mass of samples of embryos or larvae taken within ±1 day of each reported SMR.

Calculation of energy budgets and the maternal investment of each species in its eggs

Energy budgets for the entire development of each species were constructed to compare energy use between the two types of development. Energy values for biomass and oxygen consumption were calculated with data collected from two to four cultures. Biomass of eggs and changes in biomass during development were converted into units of energy, joules (J), by using the energy equivalents for combustion enthalpy for each fraction (lipid = 39.5 kJ g^{-1}, protein = 24.0 kJ g^{-1}, and carbohydrate = 17.5 kJ g^{-1}; Gnaiger, 1983). For changes in biomass, the material laid down was assumed to have the same composition as that of eggs. The composition of eggs (percent lipid, protein, and carbohydrate) of the two *Heliocidaris* species was estimated from data collected for a wide range of planktotrophic and lecithotrophic echinoderm species (table 6 in Jaeckle, 1995). These data indicate that the eggs of lecithotrophic and planktotrophic species have characteristically distinct percentages of constituents. Assuming that the remainder fraction of the egg resembles the composition of the measured portion, the mean composition of the eggs of lecithotrophic species is 57.0% lipid, 40.0% protein, and 3.0% carbohydrate and of planktotrophic species is 28.6% lipid, 65.6% protein and 5.8% carbohydrate. Our use of these estimates is supported by preliminary biochemical analyses of the composition of eggs of *H. erythrogramma;* the results indicate that larvae with a normal complement of blastocoelic lipid consist of 58% lipid, 39% protein, and 3% carbohydrate (A. Moran, unpubl. data). The composition of gonadal tissue from *H. tuberculata* and *H. erythrogramma* (Lawrence and Byrne, 1994) also supports these values estimated from Jaeckle (1995). The respective percentages of lipid, protein, and carbohydrate in tissues of the ripe ovaries were as follows: *H. erythrogramma*—50%, 38%, and 8%; *H. tuberculata*—27%, 54%, and 12% (table 3 in Lawrence and Byrne, 1994).

For each species, total metabolic expenditure for development was calculated from the total amount of oxygen taken up over the entire course of development. To-

tal oxygen consumption was determined by integrating 3rd or 4th order polynomial equations fitted to respirometry data (instantaneous metabolic rates) with a curve-fitting program (Solver, Microsoft, USA $r^2 > 0.95$). The total oxygen was converted into energy units, J, by assuming that the substrate being combusted was a mixture of lipid, protein, and carbohydrate and by using the oxyenthalpic equivalent for this mixture (480 kJ/mol O_2) determined from Gnaiger (1983).

The maternal investment of energy into the eggs of the two species was compared using calculations of the energy contained within the eggs and the energy required for complete larval development (see above). Some calculations of the energy content of the eggs of *H. erythrogramma* also took into consideration the fact that about half the egg (Table I) consists of a blastocoelic lipid-rich component that is used primarily for juvenile development (Emlet and Hoegh-Guldberg, 1997). In this case, egg energy content was calculated by excluding the proportion of the egg mass removed by centrifugation of blastulae and assuming that the rest of the egg has the same lipid, protein, and carbohydrate fractions as the original egg. This last assumption was made because the biochemical composition of the reduced-lipid embryos was not measured and will cause an overestimate of energy content of reduced-lipid embryos by as much as 15% if centrifugation produced embryos with a composition similar to that of planktotrophic eggs.

Results

Changes in dry organic mass

Heliocidaris tuberculata. Three cohorts of larvae were raised through metamorphosis with the first individuals metamorphosing on days 21, 29, and 30 for the separate cultures. Dry organic mass was measured for these cohorts and for others not raised through metamorphosis. The eggs from two cohorts of *Heliocidaris tuberculata* had estimated dry organic masses of 0.12 ± 0.01 μg egg^{-1} and 0.22 ± 0.02 μg egg^{-1} (mean $\pm$ 1 SEM), respectively. The egg masses of these cohorts are significantly different (t-test: $T = 4.82$, df $= 10$, $P = 0.001$), but the higher value for one cohort may be in error because it was based on samples frozen in small amounts of seawater and these may have absorbed moisture upon initial weighing. The value of 0.12 μg egg^{-1} falls on the regression line of egg volume and dry organic weight for eggs of echinoderm species with lecithotrophic and planktotrophic development (fig. 1 in Jaeckle, 1995) and is used for calculations throughout this study.

Larval masses increased exponentially during feeding larval development. Competent larvae, recognized by well-developed juvenile rudiments including definitive spines and shortened larval arms, had masses of 4.18 ± 0.93 μg larva^{-1} to 5.97 ± 0.34 μg larva^{-1} (mean $\pm$ 1 SEM), representing a 35- to 50-fold increase from a 0.12 μg egg^{-1} over the 21–30-day larval period (Fig. 1A). The calculated change in dry organic weight ranged between 4.06 and 5.86 μg larva^{-1}. Two-day-old juveniles of two cohorts had dry organic masses of 5.26 ± 0.24 μg juvenile^{-1} (mean $\pm$ 1 SEM) and 7.49 ± 0.39 μg juvenile^{-1}, respectively.

Heliocidaris erythrogramma. The dry organic mass of the eggs of *Heliocidaris erythrogramma* differed significantly among seven cultures (ANOVA, $F = 8.83$, df $= 6$, $P < 0.001$) and ranged from 11.59 ± 0.93 μg egg^{-1} to 18.97 ± 1.03 μg egg^{-1} (mean $\pm$ 1 SEM). Ovoid eggs had equivalent, mean spherical diameters of 369 to 418 μm for six cultures. The dry organic mass of embryos of *H. erythrogramma* from which the blastocoelic contents had been removed by centrifugation was measured to estimate the percentage of the egg mass that was contributed to the blastocoelic contents (Table IA). The mean percentage that was blastocoelic contents was 52.2 ± 5.1% (mean $\pm$ 1 SEM) and ranged from 39.8 to 64.7% (= mean $\pm$ 95% CI).

The mean dry organic masses of *H. erythrogramma* remained unchanged in one culture, increased in another, and decreased in two others after 4 days of larval development (Fig. 1B). Three of these cohorts showed nonsignificant changes in mass, and one showed a significant drop over this interval (t-tests, Table IB). Larvae of *H. erythrogramma* from which the blastocoelic lipid had been removed developed normally to the juvenile stage in the same time (3.5–4 d) as control echinoids (see also Emlet and Hoegh-Guldberg, 1997).

At metamorphosis, control juveniles of *H. erythrogramma* had substantially greater organic mass than reduced-lipid juveniles. One-day-old control juveniles from four cohorts had mean dry organic masses of 12.4, 16.5, 18.9, and 19.4 μg individual^{-1} ($n = 3$ to 6 replicate samples per cohort). Sibling reduced-lipid juveniles from the same cohorts had mean dry organic masses of 4.4, 7.2, 10.2, and 13.8 μg individual^{-1}, respectively ($n = 2$ to 5 replicate samples per cohort). These values for reduced-lipid juveniles range from 0.8 to 1.8 times the dry organic mass of juvenile *H. tuberculata.* Because metabolic studies were conducted on cohorts of *H. erythrogramma* from the upper part of the egg-mass distribution, even reduced-lipid juveniles from these cohorts had masses greater than those of *H. tuberculata.* These values indicate that juveniles of the two species differ in mass and energy content even after blastocoelic lipid materials are taken into account. See Emlet and Hoegh-Guldberg (1997) for comparisons of juvenile size and growth between the control and reduced-lipid treatments of *H. erythrogramma* and between the congeners.

Metabolic rates during development

Heliocidaris tuberculata. The metabolic rate of *H. tuberculata* increased from near zero (1.99 to

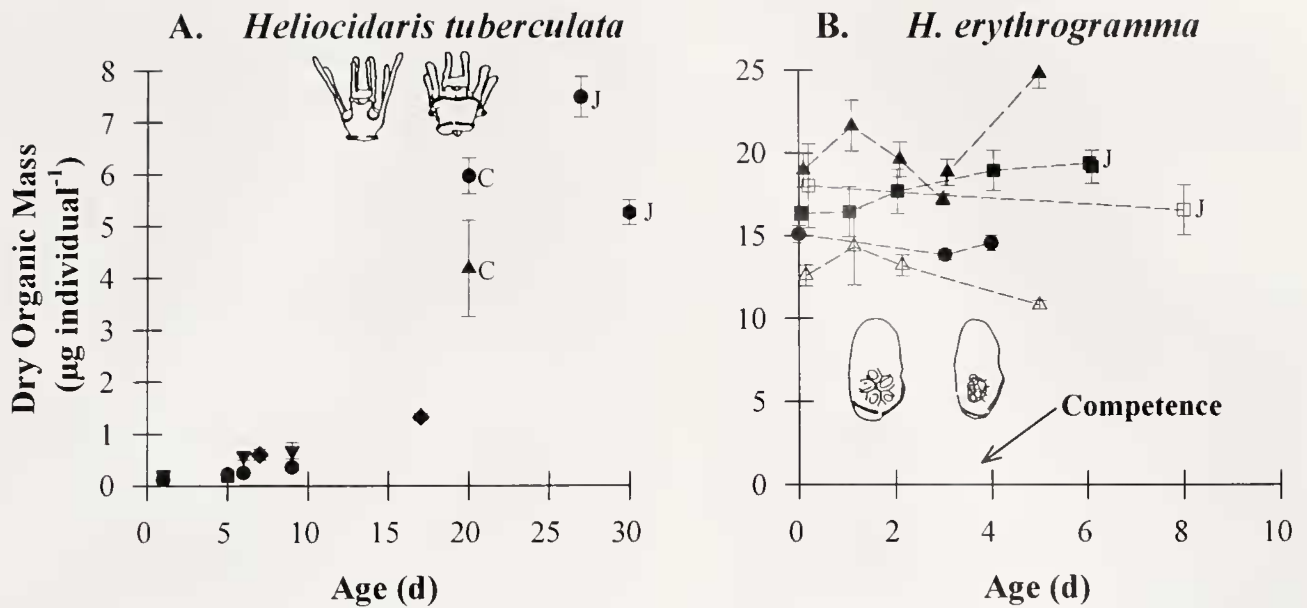

Figure 1. Changes in dry organic mass as a function of time for the embryos and larvae of the planktotrophic echinoid, *Heliocidaris tuberculata* (A), and the lecithotrophic echinoid, *H. erythrogramma* (B). Shown are means ± 1 SEM (n = 5–6 replicate weight determinations at a given age), with different symbol types representing cultures derived from separate parental pairs. Drawings indicate the morphology of larvae at various stages during development (adapted from Emlet, 1995). C, competent larval stages; J, juveniles. The arrow indicates the approximate age at which *H. erythrogramma* reaches metamorphic competence.

4.75 pmol O_2 larva^{-1} h^{-1}) to metabolic rates that ranged between 200 and 500 pmol O_2 larva^{-1} h^{-1} (Fig. 2A). Metabolic rates generally followed changes in biomass. However, specific metabolic rates (SMR) indicated that this was not strictly so. Three distinct phases could be identified (Fig. 3A): (1) An initial increase in specific metabolic rate over the first 2 days; (2) a relatively stable phase from days 2 to 10 in which the SMR ranged between 150 and 250 pmol O_2 μg^{-1} h^{-1}; and (3) a final phase (days 10–22) in which the SMR dropped to between 50 and 150 pmol O_2 μg^{-1} h^{-1}.

Heliocidaris erythrogramma. The metabolic rate of *H. erythrogramma* showed some dramatic changes during development. Metabolic rates increased steadily from 17 to 27 pmol O_2 larva^{-1} h^{-1} just after fertilization to approximately 600–800 pmol O_2 larva^{-1} h^{-1} at 36 h (22°C, Fig. 2B). A transient spike in the metabolic rate was seen in some cultures between 25 and 31 h after fertilization (corresponding to late gastrulation, early vestibule formation). This spike was not seen in all cultures, however. Metabolic rates ranged from 600 to 1000 pmol O_2 larva^{-1} h^{-1} from 1.5 days after fertilization until metamorphosis and then declined with time after metamorphosis (Fig. 2B). Two days after metamorphosis, juveniles (two cohorts), for example, had metabolic rates that ranged between 200 and 400 pmol O_2 larva^{-1} h^{-1} (Fig. 2B).

Specific metabolic rates of *H. erythrogramma* larvae were lower than those of *H. tuberculata* for most of their development (*cf.* SMR ranges, *H. erythrogramma:* 0–100 pmol O_2 μg^{-1} h^{-1}, Fig. 4; and *H. tuberculata:* 0–300 pmol O_2 μg^{-1} h^{-1}, Fig. 3A). However, if SMRs are calculated using dry organic masses from which the blastocoelic lipid fraction has been removed, SMR values showed a greater resemblance to those of *H. tuberculata* (Figs. 3A and B, 5). The metabolic rates of embryos with and without blastocoelic lipid were measured in order to investigate the metabolic activity of the blastocoelic fraction of *H. erythrogramma* embryos. Removal of lipid rich contents from embryos in five separate cultures revealed that larval metabolic rates were not different between uncentrifuged (control) and reduced-lipid embryos (Fig. 6, Nested ANOVA, testing for effects of removing lipid contents $F_{5,28} = 0.25$, $P = 0.94$).

Energy budgets, maternal investment, and the energy required to produce a juvenile

Energy budgets were constructed to provide precise measurement of the energy required for development in both species. Calculated totals were then used together with the energetic contents of eggs to provide estimates of the proportion of energy for development that was provided as maternal investment in the egg.

Table 1

Dry organic mass (micrograms per individual) for Heliocidaris erythrogramma

A. Embryos (24–30 h) with (Control) and without (Reduced Lipid) blastocoelic lipid from 7 independent cultures

	Control embryos		Reduced lipid embryos		
Culture ID (n*)	Mean	1 SEM	Mean	1 SEM	Blastocoelic Contents (%)†
HE013 (5,6)	17.7	1.4	10.5	1.3	40.8
HE016 (6,6)	18.6	1.4	11.9	1.1	35.9
HE017 (6,6)	18.9	0.8	10.2	0.8	46.1
HE94/3 (4,5)	12.4	0.5	4.4	0.5	64.3
HE94/4 (5,5)	14.4	2.3	4.4	0.8	69.1
HE94/5 (6,6)	12.7	0.7	4.4	0.3	65.0
HE94/6 (5,5)	18.0	2.5	10.0	1.6	44.4
Mean	16.1		8.0		52.2
SEM	1.1		1.3		5.1
Min (95% CI)	13.4		4.9		39.8
Max (95% CI)	18.7		11.1		64.7

B. Eggs and competent larvae from cultures shown in Figure 1B

	Eggs		Competent larvae		t-tests (df 10)
Culture ID (n*)	Mean	1 SEM	Mean	1 SEM	T, prob.
HE010 (6,6)	15.10	0.52	14.61	0.47	0.70, $P = 0.50$
HE013 (6,6)	16.35	0.42	18.96	1.21	2.03, $P = 0.07$
HE017 (6,6)	18.97	1.03	18.62	0.80	0.82, $P = 0.94$
HE94/4 (6,6)	12.59	0.64	10.85	0.25	2.53, $P = 0.03$

* The number of replicate samples for each treatment.

† The blastocoelic contents (%) is the percent of the original mass removed by centrifugation of blastulae.

Heliocidaris tuberculata. During the course of development *H. tuberculata* accumulated biomass that represented between 114 and 164 mJ individual^{-1} (Table IIA). Between 108.9 and 124.6 nmol of oxygen or 52 and 60 mJ individual^{-1} was utilized in routine metabolism during this same period (Table IIB). Excluding maternal investment in the egg, the energy for development is approximately equal to the sum of these two components and ranged between 174 mJ individual^{-1} and 216 mJ individual^{-1}. If one includes the energy invested into the egg, the energy for development is slightly higher and is equal to 177–220 mJ individual^{-1}. On a per weight basis, the energy for producing a juvenile of *H. tuberculata* ranges from 37–42 mJ μg^{-1}.

The proportion of energy for producing a juvenile that comes from maternal investment in the egg can be calculated by dividing the energy invested into an egg by the total energy for development. Values calculated in this way yielded estimates of maternal investment by *H. tuberculata* of less than 2% of the total energy required to produce a juvenile (1.3% and 1.6% for two cohorts).

Heliocidaris erythrogramma. For three of four cultures, changes in the biomass were not significant over the 3.5–4 days it took *H. erythrogramma* to develop into a juvenile (Table IB). Based on the integrated metabolic rate of *H. erythrogramma* over the time course of development, the expected decline in biomass (total energy burned in respiration divided by 27 kJ g^{-1}, the energy released per gram of a mixture of protein, carbohydrate, and lipid aerobically combusted; Gnaiger, 1983) was between 0.9 and 1.3 μg individual^{-1}. This is below the precision of the method used to measure dry organic weight.

H. erythrogramma utilized 55.2 to 74.1 nmol O_2 individual^{-1} (range of means from four cultures) to develop into a juvenile. In energetic equivalents this is 26 to 35 mJ individual^{-1} (Table IIIB). The energy required for *H. erythrogramma* to develop into a juvenile (excluding maternal investment in the egg) was therefore 29 ± 6 mJ individual^{-1} (mean ± 95% CI; Table IIIC). If the maternal investment in the egg was added (including that used for juvenile development), the total energy for *H. erythrogramma* to develop into a juvenile was 571 ± 87 mJ individual^{-1} (mean ± 95% CI; Table IIID). If one takes into account that about 52.2% of the *H. erythrogramma* egg is used for juvenile development and is not an investment in embryonic and larval development (Emlet and Hoegh-Guldberg, 1997), the energy for development is 325 ± 68 mJ individual^{-1} (mean ± 95% CI; Table IIID).

The energy for producing a juvenile was estimated by dividing the total energy for development by the mass of juvenile produced. The estimated energy for development per microgram of juvenile body produced ranged between 34.1 and 34.8 mJ μg^{-1} across the four cohorts examined. The mass-specific energy for producing a juvenile of *H. erythrogramma* was similar even when the blastocoelic contents were excluded from the calculation of both the total energy required for development and the total energy supplied to the egg (range 35.4–36.3 mJ μg^{-1}, Table IIIE).

Maternal investment including the lipid-rich blastocoelic component was 94.8% ± 1.3% (mean ± 95% confidence interval, $n = 4$) of the total energy required to make a juvenile. Maternal investment by *H. erythrogramma* excluding this blastocoelic component was 90.8% ± 1.6%. In either case this was many times greater than the maternal investment by *H. tuberculata* (<2%).

Discussion

Despite the active discussion for more than 50 years on larval life-history patterns of marine invertebrates (*e.g.*, Thorson, 1950; Crisp, 1976; Strathmann, 1985; Havenhand, 1995), precise estimates of the energetics of development are lacking for invertebrates with differing

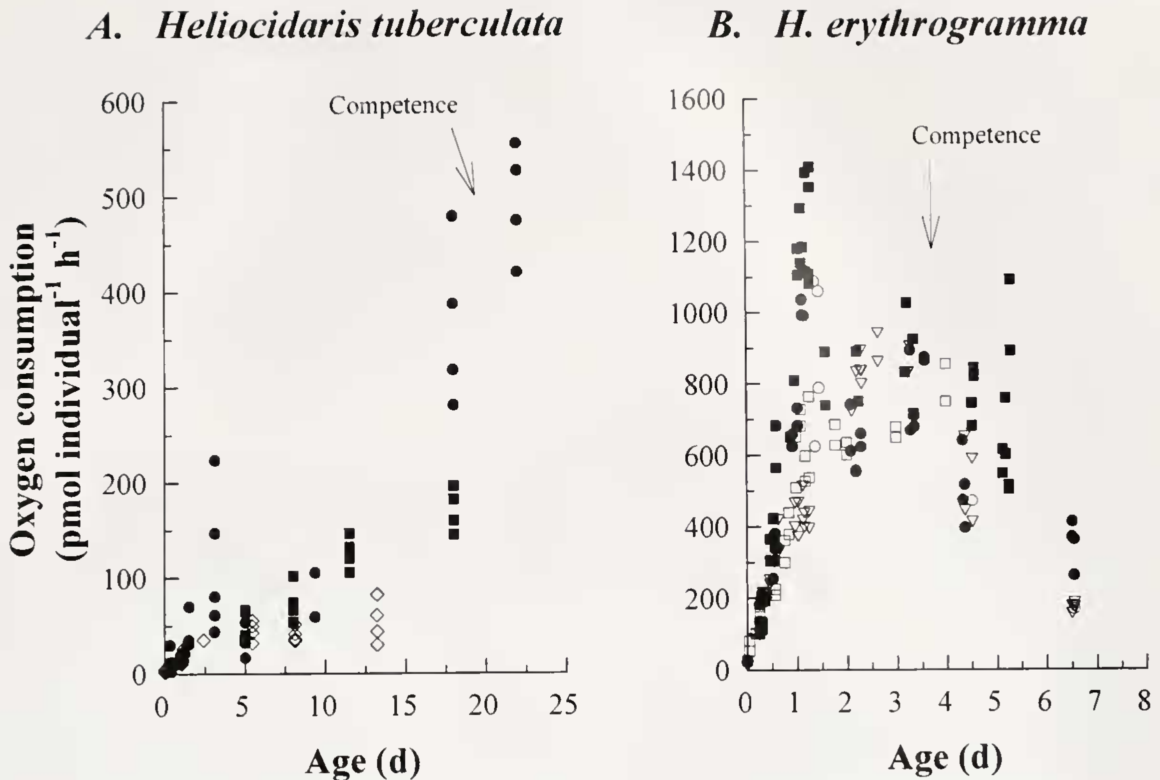

Figure 2. The oxygen consumption of embryos and larvae of the planktotrophic echinoid, *Heliocidaris tuberculata* (A), and the lecithotrophic echinoid, *H. erythrogramma* (B). Symbols represent individual respirometry measurements (a chamber of larvae at a given stage) with different symbol types representing individual cultures derived from separate parental pairs. Arrows indicate the approximate ages at which metamorphic competence occurred in each species.

modes of development. This study addresses this issue for two species of congeneric urchins, estimating the energy required for development, the energy required to build and operate a feeding larva and that required to transform an egg into a juvenile, and the percentage of the total energy required for development that is invested into the two egg types by the mother. The key observation of the present study is that although the two species differ greatly in terms of developmental mode, metabolic expenditures, and maternal investment, the energy required to make a juvenile is essentially the same when scaled to juvenile mass.

Changes in metabolic rates as a function of developmental stage

A rapid increase in the metabolic rate of both species of sea urchins characterizes the first 2 days of development. The egg rapidly differentiates into the cellular components required for further development during this period. For *Heliocidaris tuberculata*, this involves the formation of the feeding apparatus required to accumulate further resources over the 3–4-week period of development. *H. erythrogramma* develops directly into a juvenile sea urchin over 3.5–4 days at 22°C. In this case, the differentiation presumably results in a minimal swimming apparatus and the juvenile rudiment. Although comparisons are complicated by the differences in the duration of development between the two species, some interesting trends are revealed.

The rapid rise in metabolic activity slows at the end of the second day in both species and leads to a period in which the metabolic activity per gram of tissue (specific metabolic rate, SMR) remains relatively constant (Figs. 3, 4). In the case of *H. tuberculata*, the SMR during this second phase ranges between 150 and 250 pmol O_2 μg^{-1} h^{-1}. *H. erythrogramma* had SMR values ranging between 30 and 70 pmol O_2 μg^{-1} h^{-1}. Part of the difference between the SMR of the two species is due to the presence of a large amount of blastocoelic lipid in the larvae of *H. erythrogramma*. This lipid-rich store, representing about 50% of the dry organic mass of the larva, does not appear to influence the success or timing of larval development (Emlet and Hoegh-Guldberg, 1997), and its removal does not affect the metabolic activity of larvae (Fig. 6). The SMR of *H. erythrogramma* was corrected for the presence of non-metabolically active lipid by standardizing rates to masses excluding blastocoelic

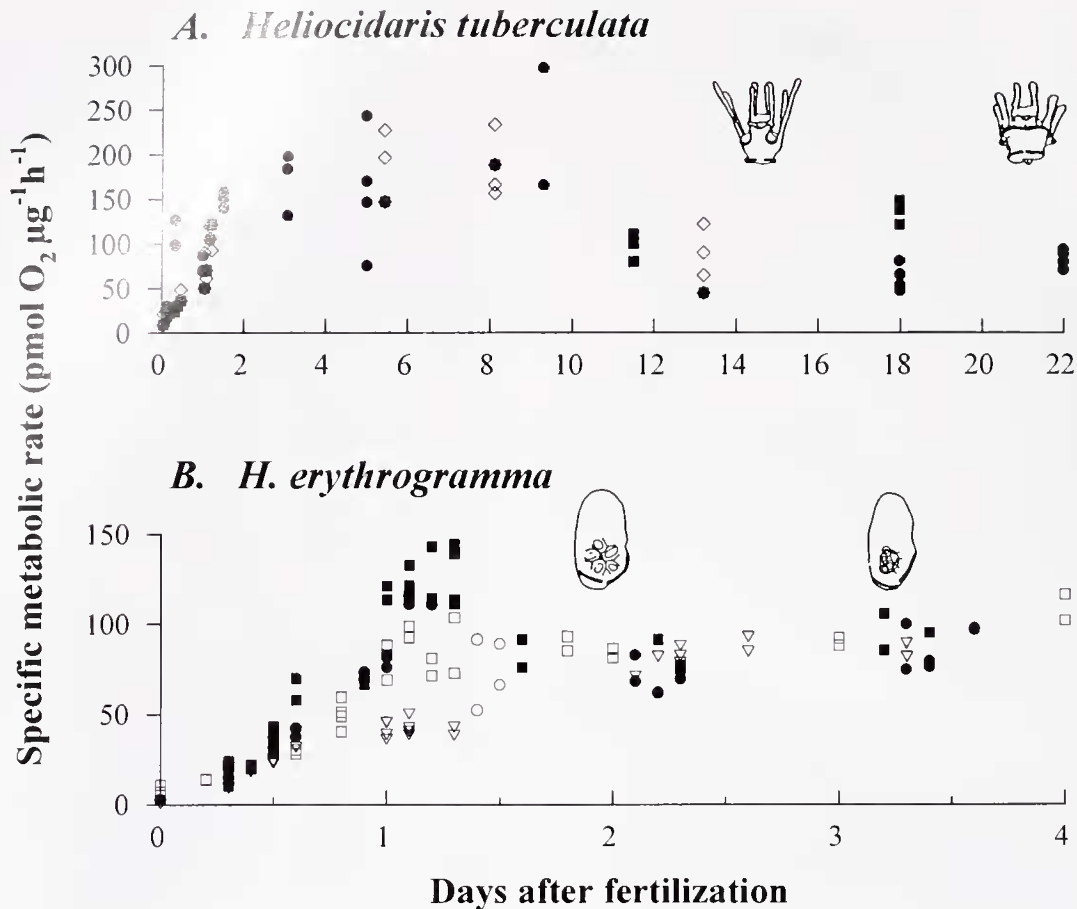

Figure 3. The specific metabolic rate of *Heliocidaris tuberculata* (A) and *H. erythrogramma* (B) as a function of age. The SMRs for *H. erythrogramma* were calculated by dividing larval metabolic rates by the dry organic mass of the reduced-lipid embryos for a given culture. This "corrected" SMR assumes that not all embryo or larval mass is metabolically active and is corroborated by data presented in Fig. 6. Symbols represent individual respirometry measurements (a chamber of larvae at a given stage), and different symbol types represent cultures derived from separate parental pairs. Drawings indicate the morphology of larvae at various stages during development (adapted from Emlet, 1995).

contents (*cf.* Figs. 3B, 4). SMR values of *H. erythrogramma* larvae corrected to active metabolic tissue were similar to the SMR of *H. tuberculata* over the last 10 days of its development (*cf.* Ht: 50–150 pmol O_2 μg^{-1} h^{-1}; He: 50–100 pmol O_2 μg^{-1} h^{-1}).

The slower and more stable growth of the feeding larva during the last part of the development of *H. tuberculata* suggests that the increased SMR earlier in the development of this species (150–250 pmol O_2 μg^{-1} h^{-1} as opposed to 50–150 pmol O_2 μg^{-1} h^{-1}; Fig. 3) is associated with the greater amount of metabolic activity required to form larval arms, skeleton, and associated feeding structures. Coeloms are also growing during this period of increased metabolism, but the vestibule or amniotic invagination has not yet formed (Emlet, pers. obs.). In this case, the difference between the larval structures of the early larval stages of *H. tuberculata* and those seen later in this species and in *H. erythrogramma* is that *H. tuberculata* does not have a significant amount of rudiment tissue early in development. This difference implies that metabolic rates of young *H. tuberculata* larvae are being standardized to smaller masses of relatively more active tissue and that rudiment tissues are relatively less active metabolically. If the SMR of rudiment tissues is lower than that of functioning larval tissues, then (in the presence of ample food or energy reserves) energetic savings might be gained by forming rudiment tissues early in development rather than investing in more expensive larval tissues.

The SMR values reported here are at the lower end of a large range of values published for feeding and growing invertebrate larvae (268–893 pmol O_2 μg^{-1} h^{-1} dry organic mass), which were reviewed by Hoegh-Guldberg and Manahan (1995), based on numbers presented by Crisp (1976). The viability of larvae during respirometry measurements was also investigated during experiments

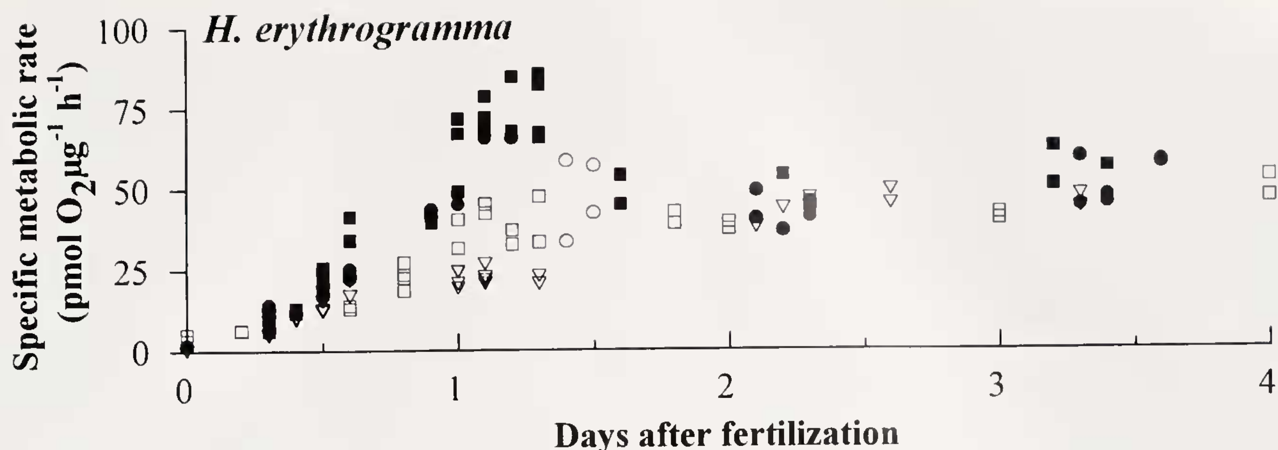

Figure 4. The "uncorrected" specific metabolic rate of the echinoid *Heliocidaris erythrogramma* standardized to the mass of the egg (including blastocoelic lipid). These uncorrected SMRs are roughly half those reported in Fig. 3B. However, the relative positions of some measurements have shifted between Figs. 3B and 4 because the % blastocoelic lipid varies among larvae from different cultures. Metamorphic competence occurred at 3.5–4 days.

reported here. Larvae were always intact and swimming normally after respirometry measurements. Ongoing work by Moreno and Hoegh-Guldberg (unpublished) has revealed that the problems reported by Hoegh-Guldberg and Manahan (1995) are probably the result of the small chambers used in the latter experiments rather than an inherent problem with the polarographic oxygen technique (leaking of KCl, effect of electric fields, etc.).

Energy budgets, maternal investment, and the energy for producing a juvenile

H. tuberculata increases in size at an average rate of between 0.20 and 0.27 $\mu g\ d^{-1}$ over its developmental period of 3+ weeks. During the same time, between 108.9 and 124.6 nmol of oxygen were consumed. By comparison, *H. erythrogramma* did not increase in size. In this case, the decrease in mass was probably in the range of 1–1.5 μg individual^{-1} (based on its metabolic rate) over its short developmental period and hence was too small to detect with the methods used here. The embryos and larvae of *H. erythrogramma* also consumed about half as much (45%–68%) oxygen as *H. tuberculata* (between 55.2 and 74.1 nmol individual^{-1}, range of means from four cultures of *H. erythrogramma*).

Although the oxygen consumption of *H. erythrogramma* was lower, its maternal investment was higher than that of *H. tuberculata*. In echinoids, the energy reserves of the egg are used by embryos and larvae as sources of both nutrients and energy. *H. tuberculata* produces eggs that are 95 μm in diameter and contain about 3.3 mJ of energy (assuming a mixture of lipid, protein, and carbohydrate that is typical of planktotrophic eggs; Jaeckle, 1995). By comparison, *H. erythrogramma* produces eggs that are 370 to 420 μ in diameter and contain between 490 and 619 mJ of energy (assuming typical lecithotrophic egg composition; Jaeckle, 1995) or 241 and 334 mJ of energy when blastocoelic lipid is excluded.

The difference in the size of egg energy reserves between *H. tuberculata* and *H. erythrogramma* contributed to the difference in energy required to make a juvenile in the two species. Results from two separate cultures revealed that it takes between 177 and 220 mJ of energy to make a juvenile of *H. tuberculata*. These values are both below the lower 95% confidence interval (257 mJ) of the energy required for making a juvenile of *H. erythrogramma* (mean = 325 mJ, see Table IIID). When the lipid-rich component is removed from embryos of *H. erythrogramma*, the resulting juveniles have test and overall dimensions at settlement that are similar to those of *H. tuberculata* (Emlet and Hoegh-Guldberg, 1997), but in some instances the dry organic masses of these juveniles were still greater than those for juveniles of *H. tuberculata*. Thus part of the absolute difference in energy for development is reflected in different masses of the resulting juveniles, even when blastocoelic lipids are excluded.

If the total energy to make a juvenile is standardized to the mass of juvenile produced, the difference between the two species largely disappears (*cf.* values for *H. tuberculata:* 37, 42 mJ μg^{-1} and *H. erythrogramma:* 34, 35, 35, 34 mJ μg^{-1} when blastocoelic contents are included and 35, 36, 36, 36 mJ μg^{-1} when they are excluded). This result means that the energy to make a juvenile *via* feeding larval development is essentially the same as the energy to make one directly from reserves added to the egg. This suggests that larval acquisition of materials from the environment to produce a juvenile may be nearly as

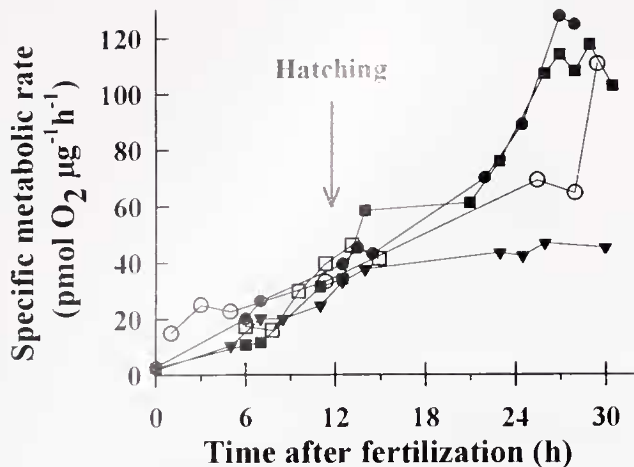

Figure 5. The specific metabolic rate of *Heliocidaris tuberculata* (open symbols) and *H. erythrogramma* (closed symbols) during early development. The SMRs of *H. erythrogramma* were calculated using masses excluding blastocoelic lipids. Symbols represent individual respirometry measurements. The arrow indicates the approximate point at which hatching occurred.

efficient as the "pre-packaging" of materials provided in the lecithotrophic egg. Comparable calculations of energy per microgram for planktotrophic larval development of the crown-of-thorns seastar, *Acanthaster planci* (from table 1 in Hoegh-Guldberg, 1994; measurements done at 27°C), and for the lecithotrophic larvae of the red abalone, *Haliotis rufescens* (from tables 2 and 3 of Jaeckle and Manahan, 1989, at 16°–17°C), yield values of 37 mJ μg^{-1} and 36–38 mJ μg^{-1} respectively, suggesting that the energy for development corrected for organic mass is strikingly similar among echinoids, asteroids, and gastropods. Further studies that include both planktotrophic and lecithotrophic comparisons are required and are in progress to substantiate this generalization and these studies (Moreno and Hoegh-Guldberg, unpublished).

The interspecific comparisons of metabolism and energy requirements allow some measure of the effectiveness with which feeding larvae acquire energy. Metabolic expenditures (measured as oxygen consumed) by larvae of *H. tuberculata* were roughly twice those measured for *H. erythrogramma* over their respective developmental intervals. Because eggs of *H. erythrogramma* contain all the necessary reserves for juvenile construction, the metabolism measured can be considered to be the metabolic cost to turn these reserves into a juvenile and maintain it (range for four cohorts = 26 to 35 mJ). If one assumes that half of the metabolic expenditure of *H. tuberculata* goes (ultimately) toward the same purpose, then the remaining half of the metabolic expenditure is used to build and power the larval feeding apparatus and digestive system to acquire these reserves. In return for this investment in larval feeding (half of the metabolic expenditure was 26 to 30 mJ individual^{-1}), the offspring increases 35- to 50-fold in organic matter, representing an increase in energy content of 114 to 164 mJ individual^{-1}. These numbers suggest that for every millijoule expended to power the feeding systems there is roughly a return of 5 to 7 mJ (1 mJ used to power the feeding systems and 4 to 6 mJ that supply materials for juvenile construction). In other words, return on the investment is 400% to 600% over the 21- to 30-day interval! These numbers may be high for several reasons. If our measurements of metabolic rates are low, then the actual investment in feeding is higher and the relative return lower. Our studies were conducted at high food concentrations under laboratory conditions. High food concentrations reduce the time for development and minimize the energy required by restricting the period over which metabolic expenditures occur. The developmental period for pluteus larvae can be extended by weeks to months on limited food (*e.g.*, Paulay *et al.*, 1985; Fenaux *et al.*, 1988, 1994; Pedrotti and Fenaux, 1993), in which time larvae are metabolically active but not gaining biomass at maximal rates (see Strathmann, 1978b, 1987, for examples of the varying planktonic durations). Under situations of food limitation with prolonged development, the return on the investment in the feeding apparatus would be expected to drop.

The values for the effectiveness of the larval feeding were based on a congeneric, interspecific study of energy for development, and we know of no similar studies for comparison. Similar measurements on other congeners with differing modes of development should permit comparisons of effectiveness of different kinds of feeding larvae. In a recent comparison of feeding rates among 11

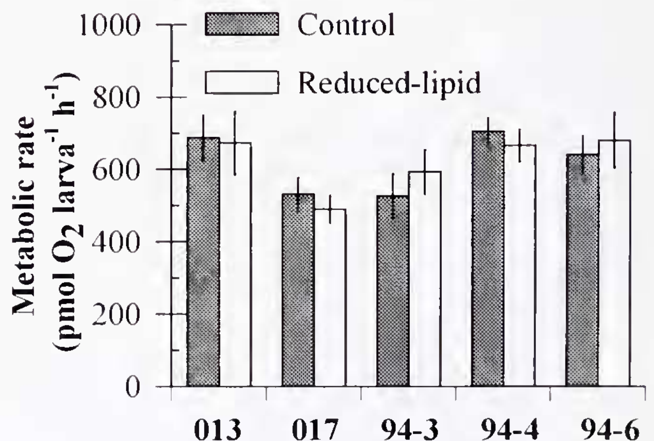

Figure 6. Metabolic rates of larvae of *Heliocidaris erythrogramma* with (control) and without blastocoelic lipid reserves (reduced-lipid). Each bar represents the mean ± 1 SEM and is based on three or four chambers of larvae. Each pair of bars is identified by a culture ID, representing separate parental pairs.

Table II

Mass and energy summaries for the entire development of Helicocidaris tuberculata *constructed from data collected from two independent cultures*

Culture ID	HT004	HT-CK1
A. Changes in biomass during development.		
(1) Biomass of egg (μg egg^{-1})	0.12	0.12
(2) Biomass at end of development (μg larva^{-1})	5.97	4.18
Biomass change (μg larva^{-1})	+ 5.86	+ 4.06
(3) Energy equivalents of biomass change (J larva^{-1})	0.164	0.114
B. Metabolic expenditure		
Total oxygen consumption (pmol individual^{-1})	108,908	124,571
(4) Energy expenditure (J individual^{-1})	0.052	0.060
C. Energy for development excluding maternal investment (J larva^{-1})		
(5 = 3 + 4) J larva^{-1}	0.216	0.174
D. Energy for development including maternal investment (J larva^{-1})		
(6) Egg investment (J egg^{-1})	0.0033	0.0033
(7 = 5 + 6) J larva^{-1})	0.220	0.177
E. Energy for development per mass of juvenile (J μg^{-1})		
(8 = 7/2)	0.037	0.042

See Materials and Methods for the basis by which biomass and oxygen consumption were converted into units of energy.

species of echinoderm larvae, representing four classes (and two body forms), Hart (1996) suggested that the pluteus body plan might be "an energetically inexpensive adaptation for suspension feeding in the plankton" (Hart, 1996, p. 42). He based his suggestion on the observations that pluteus larvae (both echinoid and ophiuroid larvae) developed and grew faster than non-pluteus larvae (asteroids and holothuroids) under similar feeding conditions, and that even though the maximal clearance rates are lower overall for the pluteus than for non-pluteus forms, the pluteus larvae also had a higher maximal clearance rate for a given number of cells in the ciliary feeding apparatus. Measurements of the energy for development of planktotrophic larvae of *Acanthaster planci* allow a partial and heuristic comparison with the values for larvae of the *Heliocidaris* species. With the same assumptions for *A. planci* as were used for planktotrophic *H. tuberculata*, the mean egg energy = 28.6 mJ, the range of energy of the added biomass = 39.3–44.3 mJ, and the range of energy expended during respiration = 16.3–29.2 mJ (calculated from table 1 of Hoegh-Guldberg, 1994). Like the values for *H. tuberculata* (Table II), the energy expended in respiration was roughly half the value of the energy equivalents calculated for the biomass added during the larval period. In the absence of a comparison with nonfeeding development in a related seastar, it is not clear how much of the respiratory costs to associate with juvenile formation versus building and running the larval feeding apparatus of the seastar larva. If we assume that these costs partition as they did for *Heliocidaris* (50% to juvenile formation and 50% to operation of the feeding system), then larvae of *Acanthaster* may experience about 270% to 540% return on investment in the feeding apparatus. This range extends lower than but overlaps the return estimated for pluteus larvae and hence is consistent with Hart's (1996) suggestion of difference in effectiveness of larval body plans. Obviously the data are limited and many assumptions are untested within this comparison. Its greatest use, however, is demonstrating the potential for inferences if appropriate comparisons are made.

Comparison of the amount of energy invested into the egg with the total energy required to produce a juvenile permits the calculation of the maternal investment in each species. *H. tuberculata* invests 3.3 mJ of organic energy into each egg; this amounts to less than 2% of the total energy needs of embryonic and larval development (177 and 220 mJ individual^{-1}, Table IIC). In comparison, *H. erythrogramma* invests between 241 and 334 mJ into each egg; this contributes 90.8% ± 1.6% (mean ± 95% CI) of the total energy required to make a juvenile *H. erythrogramma* (325 ± 68 mJ individual^{-1}, Table IIID). This study verified that a planktotrophic egg contains only a small contribution to the total energy required by a developing larva, whereas the opposite is true with a lecithotrophic egg, which makes a huge contribution to the energy for development.

Implications for the evolution of development and metabolic studies

The values for energy required to produce a juvenile imply that there are no developmentally based energetic barriers or benefits to changes in modes of development. The total energy for development (per individual) in the species with planktotrophic larvae is essentially the same as that in the species with pelagic, lecithotrophic larvae, once juvenile body size is taken into account. Selection for the increase in egg size, the loss of feeding function, and the reorganization of morphogenesis do not require increases or decreases in the overall energy required to produce a juvenile beyond those that are considered in life-history theory. The additional comparison that we offer is for the energy required for development of *H. erythrogramma* with and without the blastocoelic lipid components. This comparison not only demonstrates that this species invests materials that are not used in larval development (see also Emlet and Hoegh-Guldberg, 1997), but also corroborates the observation that similar energy for development is involved when juvenile size is

Table III

Mass and energy summaries for the entire development of Heliocidaris erythrogramma *constructed from data collected from four independent cultures*

Culture ID	HE007	HE010	HE013	HE017
A. Changes in biomass during development				
(1) Egg biomass (μg egg^{-1})	16.09	15.10	16.35	18.97
(1′) Est. biomass, excl. blastocoelic cont. (μg egg^{-1})	7.37	9.0	9.68	10.22
(2) Biomass of at end of development (μg larva^{-1})	—	14.61	18.96	18.86
Biomass change (μg larva^{-1})	—	−0.49	+ 2.61	− 0.11
(3) Energy equivalents of biomass change (J larva^{-1})*	0	0	0	0
B. Metabolic expenditure				
Total Oxygen consumption (pmol individual^{-1})	55,156	62,643	74,107	59,627
(4) Energy expenditure (J individual^{-1})	0.026	0.029	0.035	0.028
C. Energy for development excluding maternal investment				
(5 = 3 + 4) J larva^{-1}	0.026	0.029	0.035	0.028
	Mean	95% CI	Lower	Upper
	0.029	0.006	0.023	0.036
D. Energy for development including maternal investment (J larva^{-1})				
(6) Egg investment (complete egg)	0.525	0.490	0.534	0.619
(6′) Egg investment (without blastocoelic contents)	0.241	0.292	0.316	0.334
(7 = 5 + 6) Energy for development (complete egg)	0.551	0.519	0.569	0.647
	Mean	95% CI	Lower	Upper
	0.571	0.087	0.485	0.658
(7′ = 5 + 6′) Energy for development (excluding blastocoelic contents)	0.267	0.321	0.351	0.362
	Mean	95% CI	Lower	Upper
	0.325	0.068	0.257	0.393
E. Energy for development per mass of juvenile (J μg^{-1})				
(8 = 7/1) Juvenile with blastocoelic contents	0.0343	0.0346	0.0348	0.0341
(8′ = 7′/1′) Juvenile without blastocoelic contents	0.0362	0.0359	0.0363	0.0354

See Materials and Methods for the basis by which biomass and oxygen consumption were converted into units of energy.
* Changes in biomass were not significant, see Table IB.
—, no data.

taken into account. A bigger juvenile can be made with increased maternal investment, but the energy for development per individual does not change from that of a juvenile developing from planktotrophic larvae when corrected for juvenile size.

Though the overall energy for development per mass of juvenile appears unchanged, there are substantial differences in the magnitude and patterns of metabolism. Having sufficient energy reserves to fuel development appears to permit the lecithotrophic species to rapidly increase metabolic activity and sustain it at a high rate throughout development. In comparison, the metabolic rate of *H. tuberculata*, after its initial increase during embryogenesis, only increases further as larval mass and tissues are acquired. As stated before, this can take time and depends on local conditions (phytoplankton concentrations, etc.). Although these differences are in part attributable to a greater number of cells present earlier in development for *H. erythrogramma* relative to *H. tuberculata*, these patterns indicate that greater numbers of cells are metabolically active in *H. erythrogramma* soon after gastrulation.

That lecithotrophs may contain a substantial amount of metabolically inactive biomass is an important finding of this study. If this material were not excluded from our estimates of specific metabolic rate, the differences between species would be far greater. For example, the specific metabolic rates of the planktotrophic and lecithotrophic echinoid show roughly similar patterns and absolute values when inert materials are excluded from the calculations of specific metabolic rate. If we had neglected to exclude the metabolically inert blastocoelic components present in *H. erythrogramma*, however, we would have come to quite different conclusions. In that

case, the lower SMR of *H. erythrogramma* compared to that of *H. tuberculata* would have led us to conclude that specific metabolic rates are inversely related to larval size, as appears to be the case for a large proportion of the kingdom Animalia (Zeuthen, 1947). The presence of metabolically inert materials in the egg, embryos, and larvae of invertebrates is likely to be a problem in any comparison of species with differing egg size, especially in cases where the measurement and removal of these energy reserves is not as easy as for *H. erythrogramma*. Two other sea urchins, *Asthenosoma ijimai* (Amemiya and Emlet, 1992) and *Holopneustes purpurescens* (V. Morris, pers. comm; Emlet, pers. obs.), are known to extrude at least some lipid-rich material into their blastocoels, but this has not been reported for most other echinoderms with large, "yolky" eggs (*e.g.*, *Patiriella exigua*, Cerra and Byrne, 1995).

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